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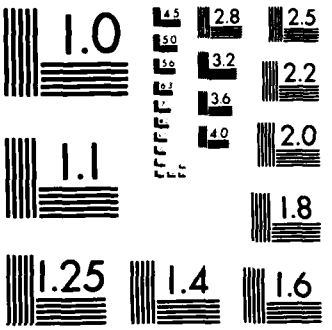

Contract No. DAMD 17-82-C-2097
William W. Monafo, M.D.
Principal Investigator

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THE FUNCTION AND STRUCTURE OF PERIPHERAL NERVES FOLLOWING CUTANEOUS BURNS

Annual Report &
Final Report

August 13, 1984

William W. Monafo, M.D.

Supported by

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An injury model which permits examination of the direct effect of transient modest elevations of tissue temperature in the region of the nerve has been developed using radiofrequency current, the effects of which are due to the heat generated. Methodology has been developed for serial clinical and electrophysiological assessment of conduction abnormalities in the posterior tibial and sural branches following this injury. In vitro electrophysiological study of nerves harvested from these animals has disclosed appreciable abnormality in the A alpha-beta. (cont)

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20. component of the compound action potential. These electrophysiological abnormalities are being correlated with morphological and biochemical changes in the nerve. In addition, some of the effects of in vivo segmental heat on conduction in peripheral nerves have been evaluated.

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An injury model which permits examination of the direct effect of transient modest elevations of tissue temperature in the region of the nerve has been developed using radiofrequency current, the effects of which are due to the heat generated. Methodology has been developed for serial clinical and electrophysiological assessment of conduction abnormalities in the posterior tibial and sural branches following this injury. In vitro electrophysiological study of nerves harvested from these animals has disclosed appreciable abnormality in the A alpha-beta component of the compound action potential. These electrophysiological abnormalities are being correlated with morphological and biochemical changes in the nerve. In addition, some of the effects of in vivo segmental heat on conduction in peripheral nerves have been evaluated.

FOREWORD:

The original proposal comprised a multidisciplinary approach to the function and structure of peripheral nerves in rat following burns. During the two years of the grant period a variety of studies within this framework were performed, the results of which are summarized in this report. This project is continuing under a research grant from the NIH # RO1 GM 3770 which began on July 1, 1984.

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Re: Contract DAMD 17-82-C-2097, Washington University, School of Medicine.

Principal Investigator: William W. Monafo, M.D.

Title: The function and structure of peripheral nerves following cutaneous burns.

INTRODUCTION:

The original proposal comprised a multidisciplinary approach to the function and structure of peripheral nerves in rat following burns. During the two years of the grant period a variety of studies within this framework were performed, the results of which are summarized in this report. This project is continuing under a research grant from the NIH # RO1 GM 3770 which began on July 1, 1984. For the purpose of clarity, the report is organized into the following divisions:

1. Clinical standardization
2. Development of percutaneous model
3. Permeability studies
4. Physiological alterations in peripheral nerves
5. Biochemical changes
6. Pathological observations

1: Clinical standardization

With rare exceptions, all studies have been carried out on Sprague Dawley rats weighing from 250 to 500 grams. It is desirable to use a single strain as variations in peripheral nerve composition (both morphological and biochemical) are known to occur between strains. There are also some age-dependent changes. Within the weight range selected, such changes are negligible, however.

After the application of percutaneous heat the neural lesions obtained in the rat necessarily vary depending on the dispersion of heat through the subcutaneous tissues and muscles and the subsequent effect on the peripheral nerves.

We wanted to be able to determine the extent of the burn induced nerve injury by clinical observation. We therefore undertook a series of evaluations of the effect of section of the three main different branches of sciatic nerves as well as of the sciatic proper. We have tested the possibility of strict clinical evaluation such as observation of the gait, of climbing

behavior, withdrawal to pin prick and analysis of foot print. It became apparent that severe burns could be easily recognized clinically from the condition of the leg, with hip flexion and protective mechanisms operating and massive swelling occurring. Less obvious motor lesions we have found to be difficult to analyze, however. Sensory function is unreliable except for the testing of pinprick in the sole of the foot, which seems to follow accurately interruption of conduction in the posterior tibial nerve. The presence clinically of toe curl, foot inversion, spreading of the toes, dragging of foot, etc. were analyzed relative to the neurophysiological findings and severity of the burn. Both surgical sectioning of the nerve and burns result in clinical lesions which are present within the first 24 hours after the animal recovers from anesthesia, but the reparative mechanisms and compensatory mechanisms are extraordinarily rapid so that 96 hours after burn or sectioning it becomes very difficult to discern a clinical motor deficit unless either the main sciatic trunk on both the posterior tibial and sural nerves are cut or blocked. (Table 1)

Electrophysiological examination of the percutaneous burn lesions have been carried out on several hundred animals. The methodology was that standardized in the literature in which electrical stimulation is applied through percutaneous platinum stainless electrodes to the proximal sciatic and to the posterior tibial at the ankle with recordings obtained from the dorsum of the foot, the injury lying between these two sites. From these studies it is clear that early interruption of conduction velocity appears in the form of conduction block in the radio frequency injury model that has been developed (v. infra). (Table 2) On some occasions in severe burns we noted an increase in terminal latency but this was not a constant finding. Edema was a complicating factor. Consistent results for the the percutaneous measurement of conduction in the peroneal nerve could not be obtained and these experiments have, therefore, been abandoned. The relation of interruption of conduction to pathological alterations will be discussed in the permeability and pathology sections.

II: Development of percutaneous models

The type of burn lesion applied were necessarily varied in the course of the investigation. Several approaches were used to develop models for assessment of effects of heat on the peripheral nerve function. Initial studies used immersion scalds; later a thermostatically controlled, heated brass block was applied to areas overlying the sciatic in the popliteal space, but this often resulted in severe injury to skin and nerve

when the temperature of the nerve in the popliteal space was only slightly elevated. This was evidently due to dissipation of the heat by circulatory and other functions. We finally settled on the use of a radio-frequency generator to permit controlled heat application and developed, in collaboration with Dr. Gilbert Nussbaum (section of Radiation Physics), several different chambers in which parallel copper electrodes were applied to the circumference of the legs or to individual nerves and heat generated using a radio frequency generator at about 1 MHz.

The fact that both immersion scalds and the contact burns to some extent are more similar to normally encountered clinical situations was considered. However, the effect of these two types of injuries on the skin, subcutaneous tissue, and muscle was severe and resulted in the animal becoming either grossly infected or attempting self mutilation. Thus we judged it more important first to determine the effect of the heat on nerve-muscle function while minimizing the skin and subcutaneous injury by irrigation of the skin with cool saline while the current was delivered to the limb. At a later stage, it may be desirable to return to the earlier models to define any additional effect of more superficial lesions. This may be particularly pertinent if superficial lesions have an effect on the development of heat and shock sensitive proteins or contribute to a remote effect on other portions of the nervous systems. Another fact on which is of major concern, as mentioned, is the effect of heat on the circulation in the leg particularly in those vessels supplying the perineurium and endoneurial structures.

A variety of prototype configurations of electrode chambers for the application of both in vivo heat loads to the exposed proximal nerve in the thigh or for percutaneous application of radio frequency current have been constructed and tested. Initially these experiments focussed on establishing a "temperature window" that could be used for the production of a reasonably fixed injury but which did not involve elevating temperature greatly beyond the physiological range. With a small operatively implanted chamber with an inter-electrode distance of 10 mm, the sciatic nerve was heated in situ with a thermocouple placed epineurially. Provided that the temperature in the epineurium did not rise above 45°C and did ot exceed four minutes, the neurophysiologically established function, as measured by conduction velocity after antegrade and retrograde stimulation, remained unchanged. No neurological deficit was observed over a period of several days. The maximal response amplitude ratio as calculated from the dorsal foot musculature was also unchanged. Light microscopic sections taken from levels

above, at and below the level of injury were normal. If, on the other hand, the exposed nerve was subjected to a temperature of 47°C plus ±.4°C for four minutes there was in every instance an immediate, significant fall in response amplitude ratio within 30 minutes. All animals subsequently had a clinically evident foot drop. Repetitive percutaneous measurements of motor nerve conduction velocity did not show significant change, provided that nerves which failed entirely to conduct an impulse were excluded. There was, however, a reduction in response amplitude to approximately 30% of normal. These results led us to the conclusion that the primary lesion produced is that of a conduction block rather than a progressive slowing of conduction velocity that is, that some fibers apparently continue to conduct at normal rates while others become nonexcitable. The conduction block could, from these observations, be selective in regard to either fiber size, fiber type, modality or geometry of the nerve. We, therefore, have conducted studies in two different directions one of which deals with the establishment of clinical percutaneous lesion, and a second which deals with the effect of in vitro heating on individual nerves and fiber types. Table III shows the response of nerve branches from normal animals to in vitro radio frequency heating.

Modifications and refinements of the percutaneous radio frequency injury model led to the manufacturing of a 20 X 25 mm copper electrodes mounted in a polycarbonate frame which permits insertion of the distal thigh and proximal calf into the chamber. The chamber is lined with sea sponge material which can be easily irrigated and the electrodes energized as previously with 1 MHz current. A percutaneously inserted temperature probe is placed in the vicinity of the sciatic nerve trifurcation. The temperature in the region of the nerve is raised to 47°C for 30 seconds. The results of such pre and post injury evaluation of nerve conduction velocity and relative amplitude of the potential evoked by stimulation at the heel and at the hip are presented in the table II. In the course of the two years we have explored the postoperative morphological, biochemical and electrophysiological characteristics of the lesions. The time post injury has ranged from 30 minutes to 96, hours with a few experiments extended longer. The data presented in Tables 5 and 6 represent values obtained at 24 hours, the time for which we have the most parallel pathological, physiological, and biochemical data. The clinical observations have been compared with those obtained from surgically interrupted nerves. Posterior tibial/sural conduction block produced by this model as measured percutaneously persists for at least 14 days.

We are of course also interested in the possibility of remote effects of the percutaneous burn injury on other nerves and in the effect of repeat injury. Changes in the in vitro parameters of the "control nerve"; i.e. the nerves in the leg opposite to that burned presented in Table 6. There are two reasons to study remote effects. One is that in experimental neuritis liberation of proteins and polypeptides with low molecular weight may result in inhibition of nerve impulse transmission. It is not known how widely circulated such proteins might be nor is it known if they may be liberated in amounts sufficient to have anything but a local effect. The second reason is the development of heat shock proteins. These proteins have a protective effect on subsequent injuries and may indeed if liberated in sufficient amount early serve to preserve conduction locally. Even considering that the percutaneous injury model must have shortcomings in that an absolutely uniform temperature elevation throughout the nerves innervating the leg cannot possibly be achieved both for geometric and circulatory reasons, it is still quite surprising that massive injury using temperatures which are known to interrupt conduction results in surprisingly minimal neurological deficits. These deficits are also quite quickly reversible. The major and serious damage appears to occur if there is massive soft tissue or non neural tissue injury. Thus one might wish to look upon part of this study as an attempt to explain the relative resilience of the peripheral nervous system to burn injury - an observation which in some ways is parallel to that made clinically in humans.

III: Permeability studies

Disturbances in permeability of blood vessels and profound alterations in blood flow are well known accompaniments of burn injuries. Permeability disturbances of the perineurium, the perineurial vessels and endoneurial vessels also occur in a number of clinical and experimental neuropathies. We have made a number of determinations of wet - dry weight ratios in normal and in burned nerves. In normal animals, distal portions of the main trunk and the proximal portions of the three major branches contain 66.01 ±4.32% water. Evan's Blue content at equilibration 20 minutes following intravenous injection is 0.273 ±.09 micrograms/mg dry weight. 24 hours following percutaneous burn injury nerve water content was increased, but no increase in Evan's Blue content was observed, the dye having been injected 20 minutes prior to nerve harvest. (Table 4) These data indicate that endoneurial permeability, by 24 hours at least, is not different from normal although residual edema (of variable amount) is persistent in the burn site and, possibly, in the contralateral side as well. Differences in injury severity could

explain the variation in the latter two variables, which we have attempted to minimize, for example, by relating water and Evan's Blue content to nerve protein content or delipidated nerve weight. Troublesome residual variation has persisted. We are currently examing the individual nerve branches and determing the time course of the apparent permeability change indicated by our morphological studies by injecting the Evan's Blue at intervals beginning immediately after injury and harvesting the branches after 20 minutes of equilibration.

More helpful in a qualitative but not necessarily in a quantitative way has been the use of Evan's Blue staining to identify areas of nerve for detailed morphological study. The pathological studies done with Evan's Blue and Lanthanum trichloride showed that substantial focal endoneurial edema appears at 24 hours. If the Evan's Blue is injected just prior to the burn, it is possible to see the most severely damaged areas and to get an impression of the distribution of the burn injury from the degree of coloration of the nerve after percutaneous burns. If the Evan's Blue is injected shortly before removal of the nerves but some time after the application of the percutaneous burn injury then an idea of the circulation and degree of collateral flow at that moment can be obtained from the staining pattern. Problems that originate with the quantitation of the Evan's Blue can be easily understood in terms of dilution of dye concentration by areas which have better collateral circulation; by complete occlusion of vessels leading to a specific, possibly large, segment of the nerve and even by an increased endoneurial pressure due to edema with subsequent even larger increase in the perineurial space and again limitation of the amount of Evan's Blue that reaches the damaged areas. We see the continued use of Evan's Blue as helpful in determining selective injury in percutaneous heating; as an indicator to areas of potential conduction block for further physiological studies and possibly a gross indicator of the effect of muscular incisions, fascial incisions and/or perineurial incisions in the management of the conduction blocks at some later phase of this study.

For the past several months we have been standarizing methodology for the determination of blood flow in the main trunk and the three major nerve branches using the hydrogen gas washout technique. Briefly, this consists of measuring tissue hydrogen concentration fall with a hydrogen-sensitive platinum electrode after equilibration has been achieved by breathing a mixture of 3% hydrogen in room air for several minutes. We will document the changes in nerve blood flow that occur at intervals following injury in the burn model and the effect of various manipulations

such as fluid therapy, fasciotomy on blood flow and nerve function.

IV: Physiological alterations in peripheral nerves

Multiple sets of experiments have been carried out in vitro in Harvard chambers on each of the three main branches of the rat sciatic nerve. In one sequence, stimulation is applied to the sciatic nerve and recordings obtained from the peroneal branch. R-F heat was applied to the peroneal nerve immediately distal to its separation from the sciatic trunk. The peroneal nerve consists of 50% motor fibers and 50% sensory fibers, the latter being large afferents from muscle spindles and tendon organs. Another set of experiments involved stimulation of the sciatic trunk heating the sural nerve branch and recording distally from the sural nerve. The sural nerve has 90% sensory fibers and only a small motor branch. The third set used the mixed motor-sensory posterior tibial nerve in similar fashion. Data obtained from the three different preparations are presented in Table 3. Sensory fibers of larger diameter appear more sensitive to heat than motor nerve fibers of the same diameter. The compound action potential of the sural nerve reflects a more pronounced decrease in the amplitude of fibers conducting at rates faster than 40 meters per second than the corresponding peroneal potential. We further tried to confirm this information by studies of dorsal and ventral root fibers and their relative behavior in response to heat. The dorsal root although ideal from the point of view of having rather pure sensory fiber composition is chemically different and its behavior cannot be directly compared with that of sensory nerve fibers. The problem will be examined further in a preparation with cut ventral roots leaving sensory nerve fibers in the posterior tibial and peroneal nerves intact. Ventral roots react to heat in a fashion similar to peroneal motor nerve fibers.

The use of paired stimuli and train stimulation at physiological frequencies have not been particularly rewarding in these preparations. One exception is the absolute refractory period. A prolongation of the absolute refractory period does occur late in the course of the heating procedure and is more pronounced in sensory fibers. It does not parallel the decrease in the amplitude of evoked response. We have not observed any changes in the relative refractory period.

Little is known about the temperature characteristics of peripheral nerve in the temperature ranges from 36°C to 45°C or 47°C. The normal temperature coefficient, Q_{10}, cannot be calculated with accuracy from the compound action potential. We

have not observed increase in conduction velocity that would be compatible with values for Q_{10} taken from other temperature ranges. It is not possible to do voltage clamp studies on single fibers at these temperatures because of the instability of the preparation. It will therefore be necessary to approach the problem of the mechanism of the earliest biophysical changes in response to heat indirectly. We have done experiments using 4 aminopyridine (4-AP) in which we show prompt restoration of an almost totally abolished potential by applying 5 mM 4-AP. As the effect of this drug is to block potassium channels, the observation can be taken to mean that the heat produces an increase in the number of potassium channels or exposes an increased area of the internodal axolemma due to damage to the perinodal region. Further studies of the potassium, sodium and calcium channels are planned for the future. We also plan approach the problem of selective impairment of large sensory fibers by exploring with microelectrodes the effect of heating on transmission of so called "natural" stimuli; i.e. vibration, tactile stimulation, heat, etc.

The physiological studies have raised some questions about whether the size of the nerve, the amount of connective tissue, the vascularity and the geometric distribution of fibers within the area recorded and/or the area heated contribute to our observations. We would want to make sure that the apparent heat resistance of the peroneal nerve that is its ability to last longer during heating to the same temperature is not because of dissemination of heat within the non-neural tissue. Other factors which we have evaluated include prolonged periods of stimulation without application of heat or prior to heating and variations in time spacing of the heat application. Neither affects the time course of the compound action potential delay. The problems of circulation have been brought up by previous authors who have justifiably felt that studies carried out <u>in vitro</u> are not directly comparable with the situation <u>in vivo</u>. The mechanism of burn injury is obviously complex and the reduction in compound action potential may reflect a combination of changes in vascular supply, electrolyte environment and direct effect on neural tissue. Other sections of this report deal with our approach to these problems.

V: Biochemical changes

Biochemical changes in peripheral nerve composition and metabolism occur in response to every conceivable type of injury. Our initial experiments compared changes in nerves that had been immersed in water for 4 minutes for 45°C as compared to 48°C. The immersed portion of the nerves were analyzed after

homogenization and removal of the lipids. Proteins obtained were separated on acrylamide gel and changes were noted between the two preparations. The changes seem to be more pronounced in the particulate fraction. We then elected to analyze the biochemical composition of the nerves at specified time intervals after the burn injury. We adapted the method described by Bell, Peterson and Wiggins 1982 to our system. In order to obtain sufficient amount of material, pools of peroneal, sural and posterior tibial nerves were prepared using 6 - 7 animals. Burn nerves and the contralateral nerves were taken from animals that had been subjected to heat 24 - 96 hours earlier. The heat applied was the same as used in our other preparations, that is 47°C at 30 seconds. After the usual percutaneous testing of conduction velocity, the nerves were removed in random sequence and stripped of epineurium. The pooled nerves were then homogenized in .29 molar sucrose and centrifuged at 100,000 X G. Soluble protein fractions were separated with trichloracetic acid precipitation and the particulate protein fraction was homogenized again in sucrose and myelin was repurified. The non myelin particulate protein was recovered through centrifugation. The crude myelin was then osmotically shocked and repurified with several centrifugation steps. Lipids were finally removed under a stream of nitrogen. The delipidated protein was then solubilized in 1% SDS and polyacrylamide gels prepared. A portion of the protein was solubilized and injected in a Beckman high performance liquid chromatograph using a TSK 3000 column 30 X .7 cm dimensions. Separation was accomplished by the use of .1 molar phosphate buffer with .1% SDS.

At 24 hours changes were observed in the myelin fraction indicating a breakdown of myelin basic protein. These changes have been confirmed in two ways. An immunological assay of the basic protein remaining in the burn nerve done in collaboration with Dr. John Trotter showed that 30 - 50% of basic protein is altered in composition. The second confirmation was obtained by immunoblot transfer and showed the appearance of several new bands which react with the specific antibody. (Fig. 1) In addition to the changes in the myelin basic protein there is also a liberation of low molecular weight protein or polypeptide bands which appear in the soluble fraction. These may be hence referred to as P_2 and P_3 or P_n and are known neuritogenic proteins. Current studies are designed to find if these also crossreact with myelin basic protein or if they can be identified by other transfer blot reactions with known antibodies.

When 96 hours have passed since the burn the changes are of slightly different character. There is still a reduction in the quantity of basic protein but the most striking change is

noted is that there is a decrease in proteins with molecular weights around 96,000 to 150,000 Daltons. (Fig. 2) These may or may not be part of the neurofilament protein triplet. We have made arrangements to have Dr. Michael Noetzel to assay these fractions in order to determine if axonal proteins are indeed destroyed by the burn. Axonal damage is certainly evident from the pathological observations: whether the changes are secondary to and specific for burn injury or if they simply reflect Wallerian degeneration as one could expect from any severe injury remains to be determined. Another avenue that we are presently exploring is the role of and presence of heat shock proteins. This particular group of proteins will be studied in collaboration with Dr. Milton Schlessinger who has the necessary antibodies available. The role of the heat shock proteins might be protective and may contribute to the rather remarkable resistance on part of some of the peripheral nerves to heat injury.

In view of the complexities encountered in determining protein changes, early studies using radio active precursors have been shelved pending identification of the more important structural changes.

VI: Pathological Changes

The pathology of the burn injured nerves have been examined in acute material immediately post burn as well as in nerves removed at 24 and 96 hours respectively from the burned animal. In addition recording sites on the nerves have been examined to determine if the nerve fiber spectrum approximately corresponds to that reported in the literature in similar rat species.

The pathological examination has included routine electronmicroscopic methodology, examination of the permeability to Evan's Blue and Lanthanum trichloride and electronmicroscopy. Teased nerve fiber preparations have been examined by both Dr. Nelson and Dr. Eliasson.

The sciatic nerve trunk proximal to the injury site, aside from occasional degeneration which could be a natural, age-determined phenomenon appear to be normal.

The peroneal nerve, examined from the junction of the peroneal with the sciatic nerve to the point were it disappears into the musculature of the interior part of the tibia, it appears to be entirely free of lesion and injection of 1% solution of Evan's Blue did not show consistent change in permeability, but again occasional degenerating axons were

observed. In teased nerve fiber preparation the paranodal region appears to be swollen with some retraction and enlargement of the node of Ranvier. These changes were progressively more prominent with increasing duration after the burn.

The posterior tibial nerve had a consistent zone of blue staining noted just distal to the junction with the sciatic nerve. Immediately post burn, disruption of both axons and myelin sheath was seen in the area heated, with axonal damage exceeding that of myelin damage. Twenty four hours post burn, there was endoneurial edema present with axonal clearing as evidenced by empty myelin sheaths and vascular congestion. Ninety six hours post burn further progression had occurred with more frank axonal loss. Leaking Lanthanum was seen emanating from the endoneurial arterials and venules 24 hours post burn. Some extravasation of red cells occurred. The thermal injury appears to have either altered the staining qualities of the axoplasm or caused the lattice to shrink and contract. In the teased nerve fiber preparations the lesions were also those of swollen paranodal areas and the appearance was dependent on which area was sampled and reflected the absence of uniform heat injury. After 96 hours, the posterior tibial was severely damaged, with evidence of general breakdown of myelin sheath and multiple fibers undergoing degeneration.

The changes in the sural nerve surprisingly enough are less pronounced than in the posterior tibial nerve but in general paralleled those just described.

Specific questions concerning the relative morphological properties are the peroneal and sural nerve which were raised because of the physiological findings can probably not be answered exactly without more extensive morphological and fiber spectrum analysis. It is, however, clear that there are no major differences between peroneal and sural nerve with regard to the relative amounts of non-neural tissue present within the endoneurium. The cross sectional area of the peroneal is indeed different and the number of axons in the peroneal nerve exceeding 8 micron in diameter is greater in our material, as reflected in the literature. Relative to the biochemical findings it has so far not been possible to demonstrate unequivocal changes in neurofilaments or neurotubules at early stages of the burn lesion.

SUMMARY:

An injury model which permits examination of the direct effect of transient modest elevations of tissue temperature in the region of the nerve has been developed using radiofrequency current, the effects of which are due to the heat generated. Methodology has been developed for serial clinical and electrophysiological assessment of conduction abnormalities in the posterior tibial and sural branches following this injury. In vitro electrophysiological study of nerves harvested from these animals has disclosed appreciable abnormality in the A alpha-beta component of the compound action potential. These electrophysiological abnormalities are being correlated with morphological and biochemical changes in the nerve. In addition, some of the effects of in vitro segmental heat on conduction in peripheral nerves have been evaluated.

MANUSCRIPTS:

1. Electrophysiological Function of Thermally Injured Rat Sciatic Nerve. Presented at the Annual Meeting of the American Burn Association, 1984. Manuscript in preparation.

2. Morphological Changes in Rat Sciatic Nerve Following Radiofrequency Burn Injury to the Hind Limb. In preparation.

3. Effect of Segmental In Vitro Heating on Rat Sciatic Nerve. In preparation.

4. Sequential Changes in Sciatic Nerve Protein Content Following in Vivo Thermal Injury. In preparation.

CLINICAL SIGNS AFTER SECTIONING OF SCIATIC TRUNK OR ITS MAJOR BRANCHS AT THE TRIFURCATION

Branch Divided	Deficit	Duration
Sciatic	Foot drop Toe curl ↓ sensation	at least 7 days
Posterior Tibial	↓ sensation sole	7 days
Peroneal	Foot drop Toe curl	≲ 4 days
Sural	? ↓ sensation thigh	---

Measurements of conduction percutaneously after the above branches were sectioned show that conduction block does not occur unless either the main sciatic trunk is divided or both the posterior tibial and sural branches are divided. This is because of a small motor branch of the sural which branches to the paw at the ankle. Immediately after the nerve is divided, complete conduction block is not necessarily demonstrable, but within a variable period of 24-96 hours, a complete block occurs.

TABLE I

CONDUCTION VELOCITY

Conduction velocity (M/Sec) rat posterior tibial nerve prior to, immediately after and 24 hours after R-F hind-limb thermal injury (temperature in popliteal space elevated to 47°C for 30 sec.) (Mean ± S.D.)

	Pre Injury	Immediately Post Injury	24 Hours Post Injury
Control Side (N=58)	65.8 ± 12.7	65.1 ± 13.4	61.5 ± 14.4
Burn Side (N=55)	61.5 ± 13.5	55.7 ± 16.4*	50.2 ± 12.7**

* not including 28 nerves with conduction block.

** not including 37 nerves with conduction block.

The data at 96 hours after injury (N=10) are similar to those shown above at 24 hours post injury. The ratio of proximal to distal maximal response amplitude in 0.83 ± 0.14 (N=46). This ratio does not consistently change following injury in nerves which still conduct, although it falls to the range of 0.25 - 0.5 in animals which eventually develop conduction block. The conduction block which appears persists for at least 14 days.

TABLE 2A

TERMIINAL LATENCY

Terminal latency (msec ± S.D.) rat posterior tibial nerve prior to, immediately after and 24 hours post R-F hind limb thermal injury (47°C X 30 Sec)

	Pre Injury	Immediately Post Injury	24 Hours Post Injury
Control Side (N = 52)	1.4 ± .19	1.4 ± .24	1.4 ± .2
Burn Side (N = 55)	1.4 ± .2	1.5 ± .3*	1.5 ± .25**

* not including 3 with no response.

** not including 27 with no response.

The terminal latency is the conduction time from the electrically stimulated area at the heel to the recording electrode over the dorsal muscles of the foot. When edema appears immediately after the heat injury, this time cannot always be accurately determined. Twenty-four hours later, excitability at the heel is frequently decreased or absent.

TABLE 28

Normals

	Peroneal	Posterior Tibial	Sural
Chamber Temperature (°C)	33.8 ± 2.3*	34.5 ± 1.24	33.8 ± 1.2
Maximal Amplitude (mV)	5.4 ± 3.1	5.77 ± 2.6	2.8 ± 1.3
Inflection Velocity (M/sec)	77.8 ± 9.0	44.6 ± 7.2	55.5 ± 6.7
Fraction Conducting at 40 M/sec or more	95.7 ± 3.3	25.4 ± 33.0	73.6 ± 21.0
End Conduction Velocity (M/sec)	34.5 ± 4.0	25.0 ± 3.3	30.0 ± 3.6
Absolute Refractory Period (msec)	0.6 ± 0.06	0.61 ± 0.1	0.54 ± 0.12
50% Decrease in Amplitude After R-F Heating to 45°C			
Rise Time (Sec)	62.8 ± 18.0	79.3 ± 8.3	61.8 ± 11.0
Time to 50% Decrease (Min)	19.1 ± 5.5	10.9 ± 5.0	12.1 ± 7.1
Maximal Amplitude (mV)	2.1 ± 1.1	2.8 ± 1.5	1.92 ± 0.98
Inflection Velocity (M/sec)	80.7 ± 5.4	38.5 ± 4.6	59.9 ± 16.4
Fraction Conducting at 40 M/sec or more	97.1 ± 2.6	3.0 ± 6.7	57.9 ± 23.8
End Conduction Velocity (M/sec)	34.4 ± 4.6	24.5 ± 2.6	30.3 ± 5.2
Absolute Refractory Period (msec)	0.59 ± 0.1	--	0.96 ± 0.23

*Mean ± S.D.

Some in vitro electrophysiological characteristics of the peroneal, posterior tibial and sural branches of the rat sciatic nerve in normals, at 34°C and after radiofrequency-induced injury over a 1.5 mm segment sufficient to reduce the amplitude of the A alpha-beta compound action potential by 50%. The time to 50% decrease in amplitude of the peroneal nerve is significantly longer than that of the sural ($P < .05$). There is also a significant fall ($P < .001$) in the proportion of fibers conducting at > 40 M/sec. in the sural nerve after heating, but not in the peroneal nerve. This raises the question whether fiber modality may be an important factor in the response of heating. This observation is being pursued.

TABLE 3

Sciatic Nerve Water and Evans Blue Content*
in Normals and on the Burned and Non-Burned Sides
24 Hours Following R-F Percutaneous Injury

	Normal	Burn	Contralateral
% H_2O	66.01 ± 4.32	69.18 ± 10.11	68.59 ± 9.30
µg Evans Blue/mg Dry Weight	.273 ± 0.09	.28 ± .06	.20 ± .09

*Injected intravenously 20 minutes prior to nerve harvest.

TABLE 4

Burned Side
24 Hour Percutaneous R-F Burns

	Peroneal	Posterior Tibial	Sural
Chamber Temperature (° C)	33.7 ± 3.4	33.8 ± 0.54	34.5 ± 2.0
Maximal Amplitude (mV)	3.6 ± 1.9	2.8 ± 2.9	2.1 ± 1.3
Inflection Velocity (M/sec)	67.6 ± 19.5	42.9 ± 25.6	52.0 ± 8.9
Fraction Conducting at 40 M/sec or more	74.5 ± 39.6	41.0 ± 34.6	46.5 ± 29.5
End Conduction Velocity (M/sec)	32.1 ± 6.4	24.6 ± 13.9	28.7 ± 4.5
Absolute Refractory Period (msec)	0.83 ± 0.46	0.58 ± 0.2	0.59 ± 0.1
50% Decrease in Amplitude after *In Vitro* R-F Heating to 45°C			
Rise Time (Sec)	66.4 ± 13.8	82.3 ± 10.9	67.3 ± 13.7
Time to 50% Decrease (Min)	10.6 ± 5.3	4.5 ± 1.9	10.0 ± 8.0
Maximal Amplitude (mV)	1.6 ± 1.1	1.8 ± 1.8	1.1 ± 0.75
Inflection Velocity (M/sec)	67.6 ± 21.7	53.3 ± 7.5	51.0 ± 10.9
Fraction Conducting at 40 M/sec or more	74.3 ± 39.8	60.8 ± 25.6	45.0 ± 34.6
End Conduction Velocity (M/sec)	31.9 ± 6.1	30.0 ± 3.3	28.6 ± 4.3
Absolute Refractory Period (msec)	0.73 ± 0.2	1.1 ± 0.1	1.0 ± 0.28

In vitro recordings from injured limbs at 24 hours. Although inflection velocity is unchanged, injured-limb nerves show much wider variability of response than do normals. The effect of an additional heat load is shown in the lower half of the table. $N \geq 10$ in all groups. Compare with normal nerves and contralateral nerves from similarly injured animals (Tables 3 and 6). Detailed, computer-assisted analysis of these data and those in Tables 3 and 6 is in progress.

TABLE 5

Contralateral - 24 Hour Percutaneous R-F Burn

	Peroneal	Posterior Tibial	Sural
Chamber Temperature (°C)	35.2 ± 2.0	34.8 ± 1.8	35.5 ± 1.2
Maximal Amplitude (mV)	4.2 ± 2.3	5.4 ± 2.7	3.1 ± 1.9
Inflection Velocity (M/sec)	68.7 ± 21.7	56.0 ± 12.2	58.6 ± 13.6
Fraction Conducting at 40 M/sec or more	70.8 ± 42.6	68.6 ± 24.0	73.2 ± 29.0
End Conduction Velocity (M/sec)	31.9 ± 6.3	29.9 ± 4.7	32.2 ± 5.7
Absolute Refractory Period (msec)	0.51 ± 0.1	0.56 ± 0.18	0.52 ± 0.06

50% Decrease in Amplitude after in vitro R-F Heating to 45°C

Rise Time (Sec)	63.7 ± 11.7	70.6 ± 19.0	54.4 ± 14.3
Time to 50% Decrease (Min)	11.9 ± 5.7	11.7 ± 5.9	8.2 ± 8.2
Maximal Amplitude (mV)	2.2 ± 1.1	2.7 ± 1.9	1.7 ± 0.96
Inflection Velocity (M/sec)	69.6 ± 25.0	53.6 ± 8.2	54.5 ± 10.3
Fraction Conducting at 40 M/sec or more	73.0 ± 38.6	59.6 ± 37.7	67.9 ± 28.1
End Conduction Velocity (M/sec)	32.8 ± 5.5	31.1 ± 4.4	29.3 ± 3.5
Absolute Refractory Period (msec)	0.72 ± 0.29	0.94 ± 0.36	0.83 ± 0.26

In vitro recordings from the uninjured limb at 24 hours. As on the burned limb (Table 5) there is considerable variance compared to normal (Table 3).

TABLE 6

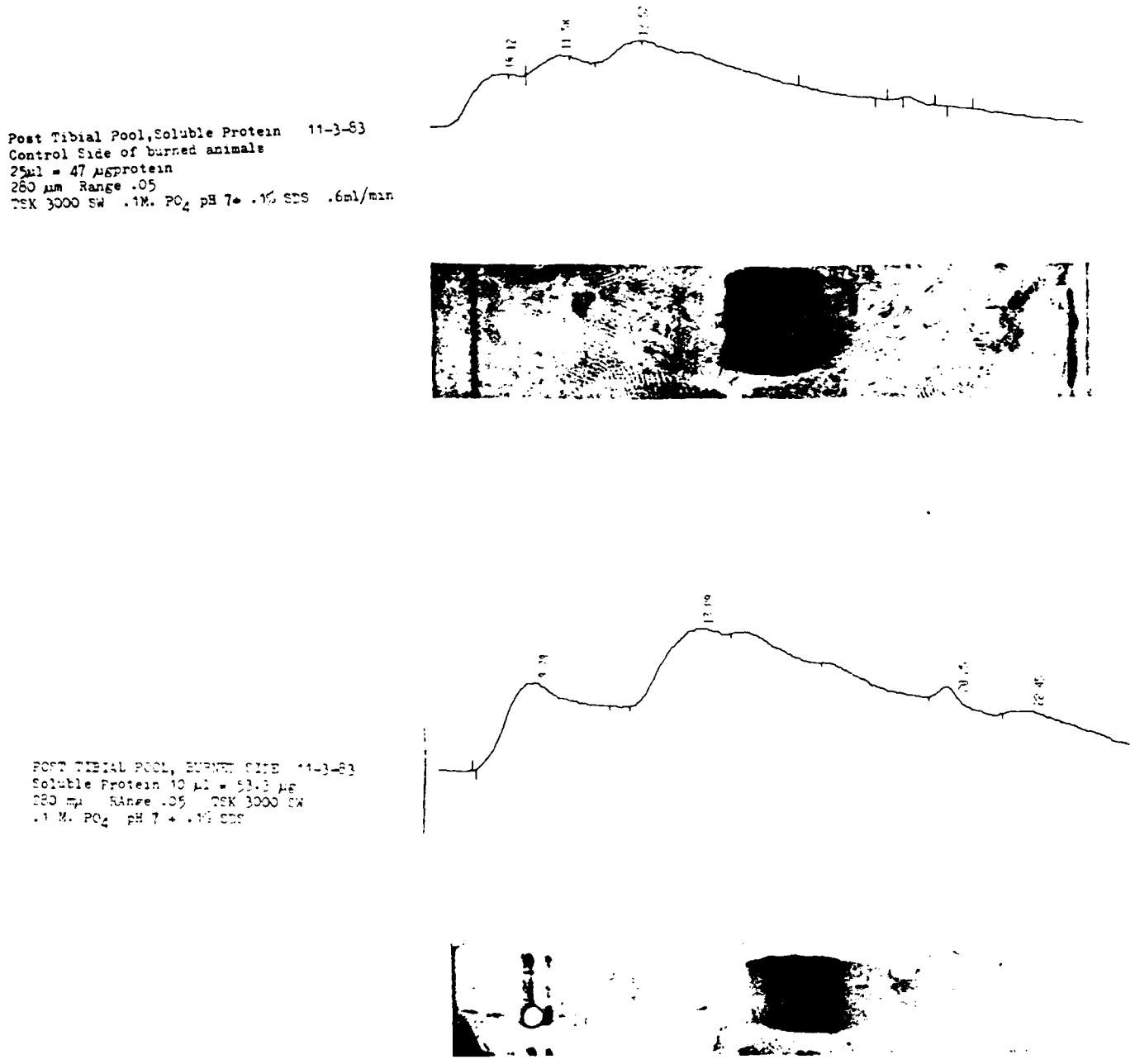

Figure 1 . Top unburned side; bottom, burned side.

Posterior tibial nerve burned 24 hours earlier (Pooled, N = 6). The soluble fraction after separation with HPLC shows several new bands. The myelin basic protein on immunoblot transfer is diminished in quantity; new high molecular weight aggregates have appeared. Some low molecular weight extension of the band is noted.

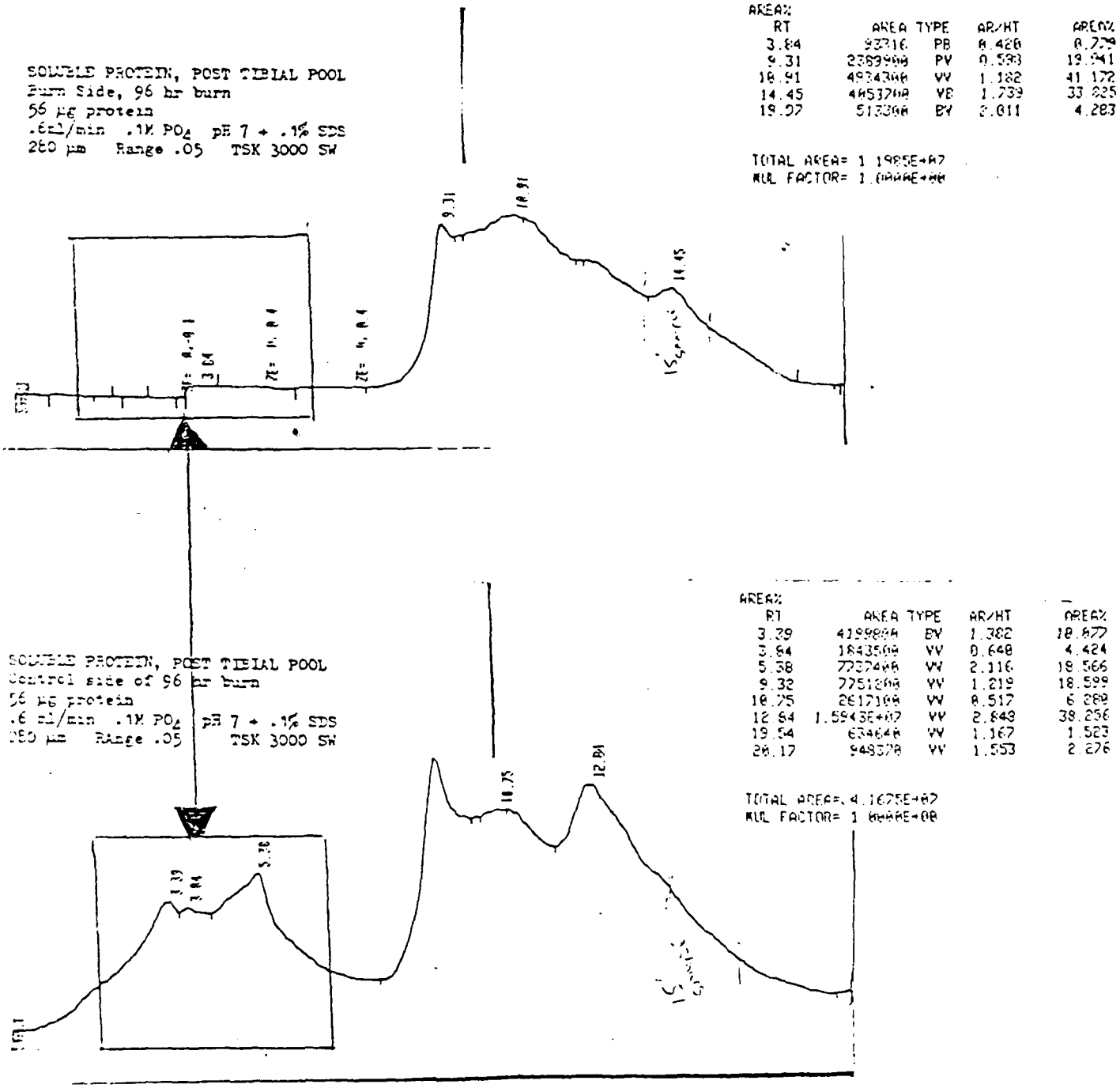

Figure 2. Pooled posterior tibial nerves (N=6) separated with high pressure liquid chromatography ninety-six hours after burn injury. The top tracing is from the burned side, the bottom tracing from the contralateral side. Within the area of the rectangles are located proteins in the molecular weight range of 150,000 to 96,000. These proteins are virtually absent from the burned nerve. This is interpreted as loss of axonal tubular and filamentous proteins.

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